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Molecular cloning of plasmolipin. Characterization of a novel proteolipid restricted to brain and kidney.

Fischer I, Sapirstein VS
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Abstract

Plasmolipin is an 18-kDa proteolipid protein found in kidney and brain, where it is restricted to the apical surface of tubular epithelial cells and to mammalian myelinated tracts, respectively (Sapirstein, V.S., Nolan, C. E., Stadler, I.I., and Fischer, I. (1992) J. Neurosci. Res. 31, 96-102; Cochary, E. F., Bizzozero, O. A., Sapirstein, V. S., Nolan, C. E., and Fischer, I. (1990) J. Neurochem. 55, 602-610). Addition of plasmolipin to lipid bilayers induces the formation of ion channels, which are voltage-dependent and K(+)-selective (Tosteson, M. T., and Sapirstein, V. S. (1981) J. Membr. Biol 63, 77-84). The present study describes the isolation and cloning of plasmolipin cDNA that includes the sequence of the complete coding region of the protein, the analysis of plasmolipin mRNA expression and a proposed model for its membrane structure. Northern blot analysis 1) shows that plasmolipin is encoded by a 1.7-kilobase mRNA, 2) confirms that the distribution of plasmolipin is restricted to kidney and brain, and 3) indicates that the expression of plasmolipin mRNA in cultured oligodendrocytes increases with cell maturation consistent with changes in the level of the protein. Restriction enzyme digestion of DNA followed by Southern blot analysis indicates that plasmolipin is encoded by a single gene. Sequence analysis of plasmolipin cDNA shows an open reading frame encoding a 157-amino acid protein of 17.4 kDa. The deduced amino acid sequence confirms the hydrophobic nature and high helical content of the protein and predicts a structure with four transmembrane domains similar to several other small hydrophobic proteins implicated in ion movement. The proposed model for membrane topology shows an enrichment of hydroxyl groups within two of the transmembrane domains and places cysteine residues near the extracellular membrane surface. Examination of protein sequence data bases reveals little overall homology with other proteins including proteolipids; however, three of the four transmembrane segments of plasmolipin show strong similarity with known membrane transport proteins. These results indicate that plasmolipin is an unique proteolipid protein that may participate in ion transport events specific to select membrane domains.

MeSH

Amino Acid Sequence; Animal; Base Sequence; Brain Chemistry; Cloning, Molecular; DNA, Complementary; Kidney; Molecular Sequence Data; Protein Conformation; Proteolipids; Rats; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

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